



# Direct quantification of inorganic iodine in seawater by mixed-mode liquid chromatography-electrospray ionization-mass spectrometry

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## ABSTRACT

Atmospheric iodine plays a relevant role in climate change. Bearing in mind that most of this iodine comes from the oceans, analytical methods capable of determining iodine in a challenging matrix as seawater are necessary. In this work, the first method capable of direct determination of total inorganic iodine in seawater at subnanomolar level based on mixed-mode liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) without any sample treatment is presented.

Analytical characteristics of the developed method were studied in terms of linear range, limits of detection and quantification, precision, trueness, matrix effect, and robustness. The detection limit for iodide was as low as 0.16 nM, injecting 5  $\mu$ L of seawater without any sample treatment and the working linear range of four orders of magnitude was wide enough to cover the broad concentration range observed in seawater samples. Average values for repeatability and intermediate precision were 4.1% and 8.1%, respectively. The suitability of the method was demonstrated through its application to the analysis of several types of samples, including seawater samples taken at different locations along the Spanish Mediterranean coast and some domestic iodized salts.

According to the results obtained, the method developed is rapid, easy to apply and to be automated, avoids sample treatment and requires only few microliters of sample. Furthermore, it has a low detection limit and allows the quantification of inorganic iodine over a wide concentration range.

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## 1. Introduction

Iodine, the less reactive halogen, can be naturally found in living organisms, Earth's crust, atmosphere, and seawater both as organic and inorganic species in multiple oxidation states. Despite its low abundance, even less than many rare earths, it is an extremely important element and object of study in physics, chemistry, biology, and medicine [1]. Apart from its biomedical importance, climate change is the other research field where the interest on iodine has increased dramatically during last years, due to its role in climate regulation. It has been demonstrated that iodine species can alter the oxidative capacity of the troposphere through a number of processes, including the depletion of ozone through catalytic cycles [2]. Most of atmospheric iodine comes from the oceans, where

iodide and iodate are its predominant species, although small quantities of molecular iodine and organoiodine compounds also exist. Thermodynamically, the stable form is  $\text{IO}_3^-$ , while  $\text{I}^-$  occurs due to the action of the biomass, especially marine algae, in such a way that up to 50% of dissolved iodine can be found as iodide in surface seawater. In fact, the ratio  $\text{IO}_3^-/\text{I}^-$  determines the redox properties of the medium, and it has been shown that there is a variation with the geographical location, seasonality, and depth [3]. The iodide content in seawater ranges from few hundreds of nanomolar to almost zero, being higher at the surface and decreasing with depth. The behavior of iodate is the opposite, since the sum of both amounts remains approximately constant, with a total concentration of 450–550 nM [3].

Therefore, quantification of both iodine species is necessary, but seawater is a challenging matrix because the concentrations of iodide and iodate extend over several orders of magnitude and, in addition, high concentrations of other anions and cations are also present, interfering the analytical measurements. These facts

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make difficult to get reliable values with most of common analytical methodologies used up to date [4,5]. Among them, electrochemical methods [6–9] are the most used due to its cost-effectiveness, high sensitivity, and low detection limits. However, these methods are cumbersome and need a high amount of sample, which has led to new analytical efforts, looking for high throughput and sample-savings methods. In this sense, chromatographic methods are particularly appropriated, as they could be capable of separating the different iodine species from matrix interferents if the separation is properly optimized. In addition, these techniques allow one to obtain quantitative results using small amounts of sample (in the microliters order).

The ionic nature of analytes and the aqueous matrix make liquid chromatography (LC) a compatible technique for the determination of inorganic iodine species, particularly using the ion exchange separation mode [10]. However, the use of LC coupled to mass spectrometry (LC-MS) is required if both high sensitivity and iodine speciation are needed. In these cases, the lack of compatibility of ion exchange mobile phases with the common electrospray ionization (ESI) source has been an obstacle to develop and popularize LC-MS methods. To avoid these problems, some authors have used ion pair chromatography [11] or have derivatized iodide and iodate [12] with the aim of using an ESI source, though with poor results. Alternatively, some successful LC-MS methods have been developed using an ICP source [13], but with little impact due to the difficulties of this ionization source handling highly saline samples.

From all above, and taking into account the importance of including iodine data in climate models to ensure greater precision and to gain a better understanding of the atmosphere [2], it is evident that an analytical method for inorganic iodine determination in seawater that could be easily implemented in common analytical laboratories would be welcome. Therefore, the aim of this work is to develop an analytical method based on LC-ESI-MS technique capable to detect iodide and iodate at nanomolar level in saline samples. The proposed method is mainly focused on obtaining better sensitivity, selectivity and throughput, and a lower sample consumption, than the methods published up to date.

## 2. Materials and methods

### 2.1. Reagents and standards

Ammonium acetate (99.9%, Sigma-Aldrich, St. Louis, MO, USA), acetonitrile OPTIMA<sup>®</sup> LC/MS grade (99.9%, Fisher Chemical, Thermo Fisher Scientific, San José, CA, USA), and ultrapure water obtained from a Milli-Q system (Millipore, Burlington, MA, USA) were used for mobile phase preparation. The pH was measured using a Thermo Orion 410A pH meter, provided with a 91-04 combined glass pH electrode with an Ag/AgCl internal reference system (Thermo Fisher Scientific). The pH meter was calibrated using aqueous buffers of pH 4.01 and 7.00.

L-(+)-ascorbic acid (99%, Alpha Aesar, Ward Hill, MA, USA) and hydrochloric acid (37%, Scharlau, Barcelona, Spain) were used for iodate reduction. Sodium chloride (99%, Panreac, Barcelona, Spain), sodium sulfate (99%, Merck, Darmstadt, Germany), sodium bicarbonate (99.5%, Riedel-de Haën, Seelze, Germany), potassium chloride (99.5%, Scharlau), sodium fluoride (99%, Sigma-Aldrich), potassium bromide (99%, Sigma-Aldrich), magnesium chloride (98%, Sigma), calcium chloride (97%, Sigma-Aldrich), and boric acid (99.5%, Sigma) were used for preparing simulated seawater (SSW), following the method proposed by Kester et al. [14].

Sodium iodide (99.999%) and sodium iodate (99.5%) used for preparing standard solutions were obtained from Sigma-Aldrich. Stock solutions were prepared in Milli-Q water and stored at  $-24^{\circ}\text{C}$

in polypropylene vials. Calibration standards were prepared daily by appropriate dilution of stock solutions in SSW.

Moreover, Triton X-100 (proteomics grade, AMRESCO, Solon, OH, USA), ammonia (25%, Sigma-Aldrich), and borate buffer pH 9.2 (boric acid 99%, Sigma-Aldrich; KCl 99%, Panreac; and NaOH 97%, Sigma-Aldrich) were used for electrochemical measurements.

### 2.2. Sample collection and preparation

To check the feasibility of the developed method, different types of samples were analyzed. First of all, samples of known content of inorganic iodine were analyzed. Thus, two iodized table salts were bought in a local supermarket. Their ingredient labels indicated that one of them was fortified with potassium iodide and the other with potassium iodate at a level of 6 mg of iodine per 100 g of salt.

In addition, seawater samples were collected from different locations along the Spanish Mediterranean coast in 100 mL polypropylene tubes that were frozen and stored at  $-24^{\circ}\text{C}$  until their analysis. Samples called Seawater 1 and 2 were collected 1000 m offshore in Salou ( $41^{\circ}04'47''\text{N}$   $1^{\circ}07'54''\text{E}$ ) and Castellon de la Plana ( $39^{\circ}58'51.9''\text{N}$   $0^{\circ}01'29.2''\text{W}$ ) beaches in summer 2017, while Seawater 3 to 8 were collected 2 m offshore in beaches of San Juan ( $38^{\circ}22'35''\text{N}$   $0^{\circ}24'32''\text{W}$ ), Almeria ( $36^{\circ}50'17.3''\text{N}$   $2^{\circ}27'35.06''\text{W}$ ), San Jose ( $36^{\circ}44'40.7''\text{N}$   $2^{\circ}7'18.501''\text{W}$ ), Nijar ( $36^{\circ}43'32.52''\text{N}$   $2^{\circ}11'42.993''\text{W}$ ), Retamar ( $36^{\circ}50'00.5''\text{N}$   $2^{\circ}19'06.8''\text{W}$ ) and Aguadulce ( $36^{\circ}48'45.2''\text{N}$   $2^{\circ}33'46.416''\text{W}$ ) in spring 2017, respectively.

All samples (100  $\mu\text{L}$ ) were filtered through a 0.20  $\mu\text{m}$  nylon syringe filter (Upti<sup>TM</sup> disk 4 mm, Interchim) and placed in insert polypropylene vials (Thermo Fisher) just before analysis. To examine the potential loss of iodide and iodate during sample filtration, a 50 nM iodide and 300 nM iodate standard was prepared and analyzed with and without filtration. The concentrations of iodide and iodate determined in the filtered standard were the same as the unfiltered standard, within the error of the method. Therefore, no losses of analytes occur during sample filtration.

### 2.3. Electroanalytical analysis

The electrochemical measurements were performed using an Ecochemie BV Autolab PGSTAT 12 with a Metrohm 663 VA Stand fitted with a static mercury drop electrode, in the hanging mercury drop electrode mode (HMDE) with the medium drop size, a Pt wire auxiliary electrode, and an Ag/AgCl/KCl 3 M reference electrode.

The evaluation of dissolved iodine speciation was carried out by cathodic stripping square wave voltammetry (CSSWV) [7]. For the direct iodide determination a deposition time of 180 s and a deposition potential of  $-0.1\text{ V}$  were used. The solution was stirred with a small magnetic stirring bar. Iodate was estimated by measuring the total iodide from the same solution by adding ascorbic acid (0.5 mM final concentration) as iodate reducer and HCl (3 mM final concentration) [9]. The reduction time was 5 min and the total iodide was measured after the pH recovery by addition of ammonia (3 mM final concentration). The solutions were prepared in 0.01 M borate buffer and none additional supporting electrolyte was necessary for the seawater samples, which were diluted with ultrapure water. KCl 0.1 M was used in all the other samples. 50  $\mu\text{L}$  of a 0.2% Triton X100 solution was added to each 10 mL of sample and the mixture was purged for 10 min with nitrogen gas (99.999% Alphagaz<sup>TM</sup>, Air Liquid, Madrid, Spain), remaining a positive nitrogen pressure throughout the measurement. The scan was from 0 to  $-0.7\text{ V}$ , with scan rate  $0.195\text{ V s}^{-1}$ , pulse height of 0.01995 V and frequency of 100 Hz. Both iodide and iodate were quantified by the standard addition method and all the results were the average of three independent measurements.

## 2.4. LC-ESI-MS analysis

LC-ESI-MS analyses were carried out using an Acquity LC system (equipped with a quaternary pump, an autosampler, and a column oven) coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, USA). The ionization source was an ESI interface working in the negative ion mode. The source and desolvation temperatures were set at 150 °C and 600 °C respectively. Ion source performance, including spray and cone voltages, was optimized infusing an 1  $\mu$ M sodium iodide solution in Milli-Q water at a flow rate of 5  $\mu$ L min<sup>-1</sup> using the on-board Xevo TQ-S fluidics system and monitoring the  $m/z$  ratio corresponding to iodide ( $m/z$  127), with the mass analyzer working in the selected ion monitoring (SIM) mode. Nitrogen (99.5% purity) was used as nebulizer (600 kPa) and desolvation (1000 L h<sup>-1</sup>) gas. The LC-ESI-MS system data acquisition and processing were managed by MassLynx 4.1 software (Waters). Some tandem MS experiments were carried out to confirm the identity of clusters observed in the full scan spectrum.

The LC separations were performed on an Acclaim mixed-mode WAX-1 column (150 mm  $\times$  2.1 mm, 3  $\mu$ m, Thermo Fisher Scientific) protected with a guard column (Acclaim Mixed-Mode WAX-1 guard column, 10 mm  $\times$  2.1 mm, 5  $\mu$ m, Thermo Fisher Scientific). The elution program was isocratic, and its flow composition was optimized as indicated in Section 3.1. The injection volume was 5  $\mu$ L, and the autosampler tray temperature was fixed at 4 °C.

Iodide was quantified injecting directly the seawater samples without any previous sample extraction nor clean-up. Total inorganic iodine was determined as iodide after its reduction following the method proposed by Campos et al. [9]. These authors concluded that final concentrations of 0.5 mM ascorbic acid and 0.03 M HCl were enough to carry out a rapid and quantitative reduction of all iodate to iodide. Thus, once adapted this method to the amounts of sample handled in this work, the procedure was as follows: to each 40  $\mu$ L of filtered seawater sample, 20  $\mu$ L of 120 mM HCl solution and 20  $\mu$ L of 2 mM ascorbic acid solution were added. Consequently, this reduces the concentration of total inorganic iodine in the sample by half and allows its quantification by interpolation into the linear dynamic working range. Samples were then vortexed for 1 min and injected into the LC-ESI-MS system. The iodate content was calculated subtracting the amount of iodide, previously obtained, to the amount of total iodine. Direct determination of iodate was not possible due to its in-source reduction (see Section 3.1). Calibration curves were prepared in SSW and all measurements were done in triplicate.

## 2.5. Method optimization and validation

Chromatographic method optimization was carried out applying a Box–Wilson Central Composite Design (BWD), which is a Response Surface Methodology (RSM). This optimization procedure is a well-established approach often used to obtain optimum chromatographic conditions and was chosen due to its widespread and versatility [15]. It is based on a factorial design together with center and start points that allow the evaluation of the relationship between a set of controlled experimental factors and observed results. Three major steps are involved in this process, namely, performing the statistical design of experiments, estimating the coefficients of the mathematical model according to Eq. (1), and predicting the response in order to check the adequacy of the model.

$$Y_{X,t} = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j + \varepsilon_{X,t} \quad (1)$$

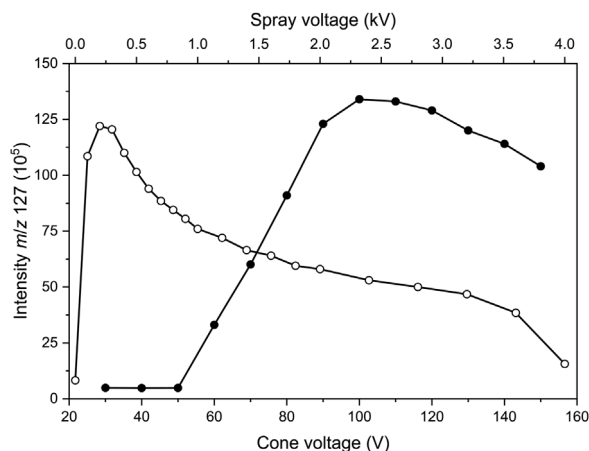


Fig. 1. Optimization curves of the cone (full circles) and spray (open circles) voltages for  $m/z$  127.

where  $n$  is the number of factors,  $Y_{X,t}$  denotes the  $t^{\text{th}}$  response observed for combination  $X = (x_1, x_2, \dots, x_n)$ , and  $\varepsilon_{X,t}$  the random-error variables. The parameter  $\beta_i$  represents the linear effect of the  $i^{\text{th}}$  factor. The parameter  $\beta_{ii}$  represents the quadratic effect of the  $i^{\text{th}}$  factor, and  $\beta_{ij}$  represents the interaction effect. The experimental design must ensure that all studied variables are examined on at least three factor levels to correctly estimate these empirical parameters.

The final analytical procedure developed was validated following the International Council for Harmonisation [16] and IUPAC [17] guidelines. Thus, the optimized method was characterized in terms of matrix effect, linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision, trueness, and robustness.

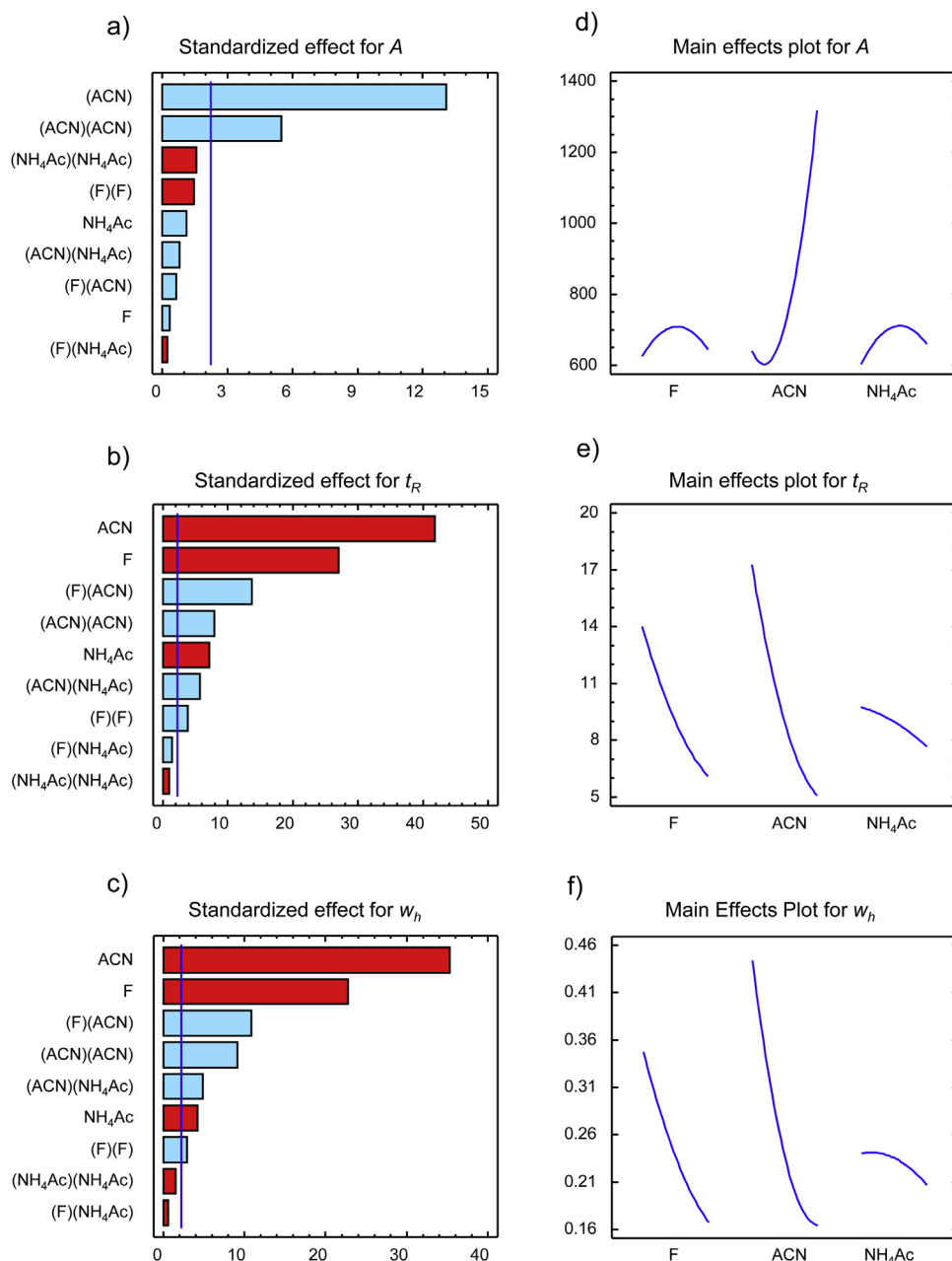
Microsoft EXCEL 2016 utility (Microsoft Corp., Redmond, WA, USA) was used for calculations and Statgraphics Centurion XV program (Statpoint Technologies Inc., Warrenton, VA, USA) for experimental design and statistical data analysis.

## 3. Results and discussion

### 3.1. Method development and optimization

The development of a new method to quantify iodide by LC-ESI-MS requires the optimization of the ionization and ion transmission in the mass spectrometer, but also of some chromatographic conditions (column temperature, eluent flow rate and composition, etc.) to ensure a mass spectrometry-friendly methodology.

Firstly, the mass spectrometric compound-dependent parameters were optimized, namely the electrospray and cone voltages. This process was carried out, as indicated in Section 2.5, by infusing a solution of iodide and working in SIM mode monitoring the intensity of  $m/z$  127. Fig. 1 shows the variation of the signal corresponding to this  $m/z$  as a function of the cone (full circles) and spray (open circles) voltages. The best results were achieved at 100 kV and 0.20 kV, respectively. It is worth mentioning that the optimum spray voltage is very low, particularly considering that the normal values are in the 3.0–5.0 kV range for organic molecules, depending on the working conditions, ion source design, and type of molecule. The reason for this low spray voltage could be the extremely acidic nature of the hydroiodic acid, with a  $pK_a$  value of  $-9.51$ . Therefore, a spray voltage of just 0.20 kV is enough to release iodide ions from the chromatographic eluent, whereas voltages beyond this optimum probably cause space-charge effects that decrease the sensitivity. Likewise, an optimization of the ESI working conditions for iodate were carried out, but with little success. During the



**Fig. 2.** Standardized Pareto charts (a–c), showing a vertical line corresponding to 0.05 of significance level, and main effect plots (d–f) obtained from the Box–Wilson design. Both graphic types are shown for A (a and d),  $t_R$  (b and e) and  $w_H$  (c and f). Bars in blue or in red indicate a positive or negative effect, respectively.

infusion of a solution of iodate ( $m/z$  175), a very intense peak at  $m/z$  127 was detected, indicating its in-source reduction to iodide. This reaction prevented the direct determination of iodate at nanomolar level, and forced its quantification as iodide (Section 2.5).

An Acclaim mixed-mode WAX-1 chromatographic column was chosen to carry out the separation. This column is packed with a silica-based stationary phase that incorporates a hydrophobic long alkyl chain with a tertiary amine terminus, providing a multimode separation mechanism that includes hydrophobic interactions, anion exchange, and cation exclusion. As a result, the retention of basic, acidic, and neutral molecules can be independently or concurrently adjusted by changing the pH, ionic strength, and organic solvent content in the mobile phase. Besides, this column allows the use of mobile phases suitable for ESI-MS analysis by choosing appropriate volatile modifiers, and has been successfully applied to analyze organic and inorganic anions [18,19]. Therefore, the

Acclaim mixed-mode WAX-1 was selected as a very convenient column to develop a LC-ESI-MS method for the analysis of iodide in seawater samples. Once the column was chosen, a series of exploratory experiments were performed to find the most relevant working parameters, which will be later used in the optimization of the chromatographic process by experimental design. Different organic modifiers (methanol and acetonitrile), additives (ammonium formate and ammonium acetate) and their pH values (3.0, 4.7 and 6.0), elution modes (gradient and isocratic), and column temperatures (30, 35 and 40 °C) were tested. According to the results, ammonium acetate at pH = 6.0 was used as additive, and acetonitrile was selected as organic modifier because gave better peak shapes and lower back pressure than methanol. The chromatographic runs were carried out in isocratic mode, and the column temperature was set at 35 °C.



**Table 1**

Chromatographic factors used for the Box–Wilson Central Composite Design (see text for symbol meaning).

Level	$F$ ( $\mu\text{L min}^{-1}$ )	ACN (% v/v)	$\text{NH}_4\text{Ac}$ (mM)
–	200	50.0	7.5
0	365	65.0	11.25
+	530	80.0	15.0

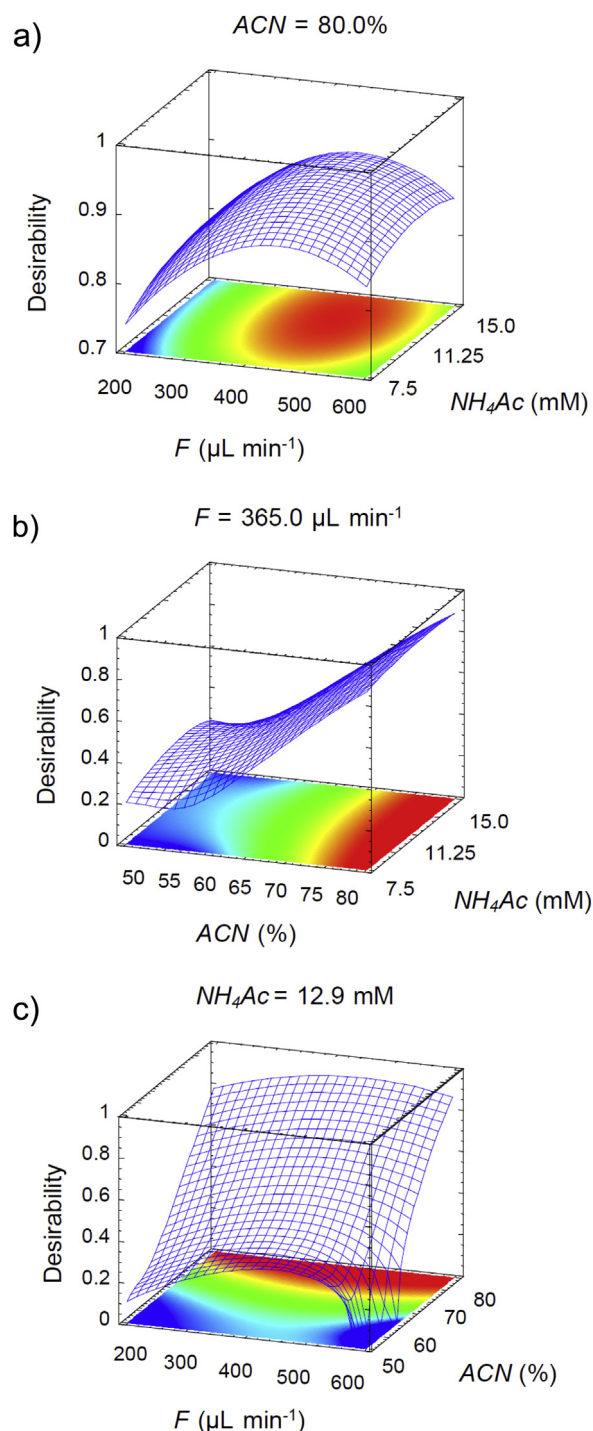
Next, the elution conditions were optimized by a Box–Wilson Central Composite Design (BWD) using the mobile phase flow rate ( $F$ ,  $\mu\text{L min}^{-1}$ ) and its final contents of acetonitrile (ACN, %) and ammonium acetate ( $\text{NH}_4\text{Ac}$ , mM) as factors. Table 1 shows the levels used for each factor. The aim of this BWD was to achieve the highest sensitivity with the lowest analysis time, so a multiple optimization was carried out using the desirability function ( $D_f$ ) [20]. The peak area of iodide ( $A$ ) was taken as a measure of sensitivity, while its retention time ( $t_R$ ) and its peak width at half height ( $w_h$ ) were selected as indicators of analysis time and chromatographic performance, respectively. Experiments carried out for the BWD, including the corresponding factors, responses, and  $D_f$  values are shown in Supplementary Table S1. On the other hand, the degree of influence of each variable and the second order effects can be observed in the Pareto charts resulting from the BWD gathered in Fig. 2. Blue and red bars stand for positive or negative effects, respectively, and the vertical line represents the significance level ( $\alpha$ ) of 0.05. The only variable with a significant effect on sensitivity at  $\alpha = 0.05$  is ACN and its second order interaction (Fig. 2a and d). The effect is positive, so that the iodide peak area increases considerably with ACN, though no absolute maximum is observed in the range of compositions studied. The effects on analysis time (Fig. 2b and e) and chromatographic performance (Fig. 2c and f) are both similar and much more complex. There is a negative effect of ACN,  $F$  and  $\text{NH}_4\text{Ac}$ , apart from a number of second order interactions. In none of these cases a global maximum or minimum has been found.

The response surfaces are gathered in Fig. 3, showing a smooth behavior into the ranges considered, which favors the robustness of the method. The optimum area is located at high values of ACN and intermediate values of  $F$  and  $\text{NH}_4\text{Ac}$ . The good quality of the BWD is indicated by the high  $D_f$  value (0.92) and the good agreement between the predicted and the observed parameters, with a maximum difference of 5.3% for  $w_h$  (Supplementary Table S1). Therefore, the optimal conditions were reached in isocratic elution mode at  $35^\circ\text{C}$ , with a flow rate of  $450 \mu\text{L min}^{-1}$  and a mobile phase composition of 80% acetonitrile, 20% ammonium acetate 64.5 mM at pH = 6.0 (which involves a final concentration of 12.9 mM in the whole mobile phase).

### 3.2. Method validation

The developed LC-ESI-MS method was validated following the ICH [16] and IUPAC [17] recommendations, using simulated seawater samples prepared according to Kester et al. [14].

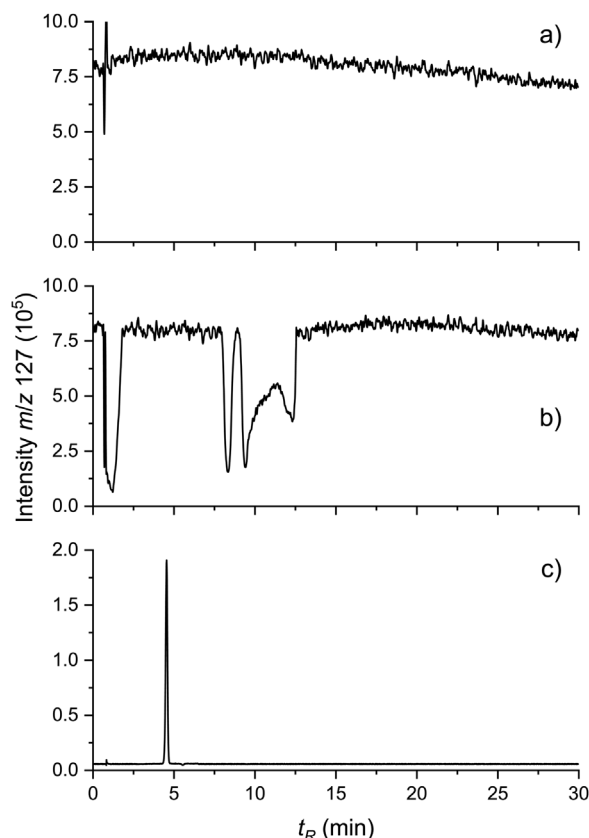
The existence of matrix effect and its dependence on the elution time was verified by the widespread procedure of infusing a standard solution while an injection of matrix is running [21]. In this case, a standard of iodide (100 nM) was post-column infused using the on-board Xevo TQ-S fluidics system, and  $5 \mu\text{L}$  of Milli-Q water or SSW were injected while monitoring the  $m/z$  127. The profiles are shown in Fig. 4, where the decrease of signal intensity for  $m/z$  127 reveals important inhibition zones at several retention times when SSW is injected (Fig. 4b), but not when the sample is Milli-Q water (Fig. 4a). The first inhibition zone was situated between 0.7 and 2.0 min and dominated by the  $m/z$  141, which was identified by MS/MS experiments as an acetate-sodium cluster, namely  $[\text{Na}(\text{CH}_3\text{COO})_2]^-$ . Later, two large negative peaks appear at 8.3 and



**Fig. 3.** Response surface plots corresponding to the desirability function ( $D_f$ ) against all the possible couples of variables used in the Box–Wilson design, maintaining one of the variables at optimum values.

9–12.5 min, corresponding to the elution of  $\text{Br}^-$  ( $m/z$  79, 81) and  $\text{Cl}^-$  ( $m/z$  35, 37), respectively. Moreover, it is important to point out that, if the post-column infusion is stopped and a 10 nM iodide standard is injected, iodide elutes in a steady zone free of relevant inhibition effects (Fig. 4c).

In order to quantify any minor matrix effect, three different calibration curves were prepared, two of them using Milli-Q water and SSW as solvents, and the third one by the standard addition method in the real seawater sample referred as Seawater 1 (Supplementary Fig. S1). Slopes of SSW and standard addition calibrations are very



**Fig. 4.** Intensity profiles of  $m/z$  127 obtained for the post-column infusion experiment when Milli-Q water (a) or SSW (b) is injected into the analytical column. Chromatogram of a 10 nM iodide standard (c).

similar, showing no significant differences ( $p < 0.05$ ). However, they are a 9.7% lower than the slope of calibration made using Milli-Q water. These results reveal two facts, firstly, the lower slopes of SSW and standard addition calibrations imply the existence of a small but significant matrix effect when samples are saline; and secondly, SSW can be used as surrogate of real seawater samples for iodide quantification. Therefore, the analytical method characterization was carried out using SSW as solvent, in order to minimize the bias of the procedure.

The peak obtained for iodide with the optimized LC-ESI-MS method (Fig. 4c) shows a narrow and fairly symmetric ( $A_s = 1.10$ ) peak, well resolved from inhibition zones. Therefore, the chromatographic separation, together with the specificity of mass spectrometric detection, make this method selective enough to carry out a reliable quantification of inorganic iodine in seawater.

The linear range was calculated from a calibration curve made injecting standards of iodide in triplicate at 16 concentration levels, between 0 and 1000 nM. A curved behavior was evident (Supplementary Fig. S2c).

The limits of detection (LOD) and quantification (LOQ) were calculated as  $3s_b/m$  and  $10s_b/m$  where  $s_b$  is the peak area standard deviation of a 0.2 nM iodide standard injected six times and  $m$  the calibration slope. Thus, the values obtained for LOD and LOQ were 0.16 and 0.49 nM, respectively. A comparison among the analysis methods found in the literature for direct determination of iodide is shown in Table 2, where only the studies with the lowest LOD values have been chosen. The LOD value for the LC-ESI-MS method proposed in this work is at the same level than the well-established electrochemical (CSSWV) methods [22,23], but with a much lower consumption of sample. Furthermore, the CSSWV methods are difficult to automate and the quantification should be carried out by

the standard addition procedure. On the other hand, flow injection analysis [33,34] is no competitive because gives relatively high LOD values and needs large volumes of sample. Electrophoretic methods [25,27] require low volumes of sample, but also fail to reach subnanomolar LOD values. Methods based on chromatographic separations are the most widespread, probably because they are very robust and easy to automate. Ion exchange is the prevalent separation mechanism, although other modes such as size exclusion and partition have also been applied. However, only those methods using inductively coupled plasma and mass spectrometry have LOD values similar to the values reached by the method proposed in this paper.

Precision was assessed by repeatability and intermediate precision at three different concentration levels of iodide (5, 25, and 100 nM in SSW). Repeatability was estimated by the relative standard deviation (RSD, %) of the peak areas obtained by injection of six aliquots of an iodide standard in SSW in the same day. Intermediate precision was evaluated by the RSD of the iodide peak area obtained in six different days over six months, using two analysts, several batches of reagents and two chromatographic columns. The RSD values for iodide repeatability were in the range 3.0–5.5%, and for intermediate precision were between 4.0 and 13.1% (Supplementary Table S2) can be considered really satisfactory.

As stated in Section 2.5, the developed method quantifies iodide directly, but the concentration of iodate is calculated as difference between iodide and total inorganic iodine, which is determined after dilution and sample reduction with ascorbic acid at pH=2. Therefore, a recovery study on iodate reduction process was carried out using three sets of six samples of SSW spiked at 50, 150 and 400 nM of  $\text{IO}_3^-$ , respectively. According to the results obtained, the reduction was very efficient with an average recovery value of 101.8% for 50 nM, 101.1% for 150 nM and 100.3% for 400 nM spiked samples.

The robustness of the method was assessed by applying a saturated factorial fractional design of experiments [36], because many factors can be studied with just a few experiments. The experimental factors selected were the same as in the BWD, but also including other parameters that could affect the quality of results, such as the pH of the buffer solution (pH) and the column temperature ( $T_c$ ). Likewise, the variables monitored were the same as in the BWD, that is peak area ( $A$ ), peak width ( $w_h$ ) and retention time of iodide ( $t_R$ ). The experimental factors as well as the chosen levels, and the investigated responses together with their experimental values are in Supplementary Tables S3 and S4, respectively. Pareto graphs (Supplementary Fig. S3) indicate that the mobile phase pH and its acetonitrile content (ACN) are the main factors influencing these variables. Peak width is also affected by the ammonium acetate ( $\text{NH}_4\text{Ac}$ ) content. These results show that the developed method can be considered robust, as the specifications of current LC instruments show maximum expected errors for the working parameters well below the considered for the robustness study. Apart from this, the stability of the mass spectrometric signal was also monitored, checking how the sensitivity (calibration slope) changed during a continuous series of analysis. The results confirmed the robustness of the method, as the maximum difference found between 5 calibrations carried out over a period of 24 h was just of 4.9%.

Finally, the effect of seawater salinity on quantification was specifically studied because this parameter could have a relevant influence on the applicability of the optimized method. As salinity of seawater ranges from 3.1 to 3.8%, three sets of SSW samples with a salinity of 3.0, 3.5 and 4.0%, and spiked at 50 nM with iodide were analyzed. Mean values and standard deviation of five injections were compared by an ANOVA, and no significant differences were found at the 95% of confidence level (see Supplementary Fig.

**Table 2**

Comparison of analytical methods for direct determination of iodide in seawater.

Technique	Separation mechanism	LOD (Iodide, nM)	Sample injection volume	Reference
CSSWV <sup>a</sup>	–	0.15	5,000 µL <sup>f</sup>	[22]
CSSWV	–	0.08	2,000 µL <sup>f</sup>	[23]
LC-UV	Ion exchange	3.94	500 µL	[24]
tITP-CE-UV <sup>b</sup>	Electrophoretic migration	1.57	7 s <sup>g</sup>	[25]
LC-UV	Ion exchange	3.00	140 µL	[26]
tITP-CE-UV	Electrophoretic migration	3.15	10 s <sup>g</sup>	[27]
LC-Voltamperometry	Ion exchange	3.94	50 µL	[28]
LC-ICP-MS <sup>c</sup>	Ion exchange	15.8	10 µL	[13]
LC-UV	Ion exchange/Partition	26.0	25 µL	[29]
LC-ICP-MS	Size exclusion	0.24	20 µL	[30]
LC-UV	Ion exchange	12.6	200 µL	[31]
LC-ICP-MS	Partition	0.41	20 µL	[32]
FIA-MS <sup>d</sup>	–	37.0	190 µL	[33]
FIA-FL <sup>e</sup>	–	2.36	350 µL	[34]
LC-UV	Ion exchange	9.45	125 µL	[35]
LC-ESI-MS	Ion exchange/Partition	0.16	5 µL	This work

<sup>a</sup> CSSWV, Cathodic stripping square wave voltamperometry.<sup>b</sup> tITP-CE, Transient isotachopheresis-capillary electrophoresis.<sup>c</sup> ICP, Inductively coupled plasma; <sup>d</sup> FIA, Flow injection analysis.<sup>e</sup> FL, Fluorescence.<sup>f</sup> Corresponds to the volume of sample used for the measure.<sup>g</sup> Represents injection time.

S4), so the developed method can be safely applied to seawater samples from any geographical location.

Summarizing, all the data presented for method development and validation demonstrate the reliability of the presented method and its advantages compared from previously published methodologies, i.e. it avoids any sample treatment, it is easier and faster than electrochemical methods, it reaches lower LOD than flow injection analysis, and it uses just a few microliters of sample (far below the rest of published methods).

### 3.3. Application of the new LC-ESI-MS method to saline samples and comparison with its electrochemical determination

The applicability of the LC-ESI-MS method optimized in this work was evaluated by determining the content of iodide and iodate in different saline samples. The selected samples included one SSW sample spiked with iodide (50 nM) and iodate (350 nM), two commercial iodized table salts (one iodized with potassium iodate and the other with potassium iodide) and eight seawater samples taken at several locations of the Spanish Mediterranean coast (see Section 2.2 for details). Taking into account the low LOQ value of the method developed, and in order to extend the chromatographic column lifetime and avoid overloading, a lower injection volume (1 µL) was used for analyzing the table salts and seawater samples.

Since natural salt contains very low levels of iodine, salts used for human nutrition are fortified with iodide or iodate. According to the Spanish regulation, iodized table salts should contain 60 mg Kg<sup>-1</sup> of iodine with a maximum accepted deviation of 15%, so solutions at an expected concentration of 48.5 nM iodate (Table salt 1) and 49.0 nM iodide (Table salt 2) were prepared and measured by the LC-ESI-MS method. Table 3 gathered the results obtained for these samples, and also for the SSW and the seawater samples. The content of iodide and iodate determined for the three samples of known concentration (SSW, Table salts 1 and 2) agrees with the expected values, with a maximum difference of 1.2% and a mean value of 0.8%, confirming again the low bias of the LC-ESI-MS method.

A representative group of these samples was also used for testing the analytical trueness of the new method by comparison with values obtained using the electrochemical method developed by Campos et al. [9]. This one has been selected due to its low LOD, and also because it is, by far, the most used to characterize the

**Table 3**

Iodide and iodate mean concentrations (n = 3) found in saline samples, and its standard deviation.

Sample	LC-ESI-MS method				CSSWV method			
	[I <sup>-</sup> ] (nM)		[IO <sub>3</sub> <sup>-</sup> ] (nM)		[I <sup>-</sup> ] (nM)		[IO <sub>3</sub> <sup>-</sup> ] (nM)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SSW <sup>a</sup>	49.4	3.5	347.7	19.7	50.8	1.5	344.0	4.8
Table salt 1 <sup>b</sup>	N.D.	–	48.9	3.3	N.D.	–	46.5	1.6
Table salt 2 <sup>c</sup>	49.3	3.3	N.D.	–	52.5	2.1	N.D.	–
Seawater 1 <sup>d</sup>	48.6	2.5	372.4	13.9	50.7	1.8	369.8	11.2
Seawater 2 <sup>d</sup>	64.8	4.6	317.9	11.7	68.4	0.9	315.4	12.1
Seawater 3 <sup>e</sup>	137.6	8.0	375.9	11.7	137.9	3.5	366.0	9.9
Seawater 4 <sup>e</sup>	166.0	10.0	329.2	20.4	159.3	4.8	334.7	6.0
Seawater 5 <sup>e</sup>	141.7	9.1	340.7	13.2				
Seawater 6 <sup>e</sup>	124.2	7.9	349.8	9.6				
Seawater 7 <sup>e</sup>	142.0	8.4	364.5	11.3				
Seawater 8 <sup>e</sup>	139.1	6.7	347.8	15.2				

<sup>a</sup> Simulated seawater sample, 50 nM iodide and 350 nM iodate.<sup>b</sup> Iodized table salt sample, 48.5 nM iodate.<sup>c</sup> Iodized table salt sample, 49.0 nM iodide.<sup>d</sup> Seawater sample taken 1000 m offshore in summer.<sup>e</sup> Seawater sample taken near shore in spring.

inorganic iodine content of seas and oceans. Results are gathered in Table 3, and show that the average concentrations obtained by using CSSWV and LC-ESIMS methods are very similar, although the standard deviation of the chromatographic method is higher in all cases. An ANOVA (data not shown) confirmed that there are no significant differences between concentration values obtained by both methods at the 95% of confidence level. To check this point, a linear regression analysis was carried out using these concentration values as variables (Supplementary Fig. S5). The model obtained was:

$$[I^-]_{\text{LC-ESI-MS}} = -1.8(\pm 2.1) + 1.014(\pm 0.009)[I^-]_{\text{CSSWV}}$$

$$R_{\text{adj}} = 0.9996; F = 12490; s_y = 4.26; n = 12$$

where  $R_{\text{adj}}$  is the correlation coefficient adjusted for degrees of freedom,  $F$  is the Fisher's factor,  $s_y$  is the standard error of the estimate, and  $n$  is the number of solutes included in the model.

As can be seen from the statistics, there is a significant linear relationship between  $[I^-]_{\text{LC-ESI-MS}}$  and  $[I^-]_{\text{CSSWV}}$  values. In addition, to consider that the new method produces values equal to



those obtained by the electrochemical method, the intercept of the model should be equal to 0, and the slope should be 1. As the estimated lower (LCL) and upper (UCL) confidence limits (at the 95% confidence level) for intercept ( $LCL_{\text{intercept}} = -6.5$ ,  $UCL_{\text{intercept}} = 3.0$ ) and slope ( $LCL_{\text{slope}} = 0.994$ ,  $UCL_{\text{slope}} = 1.034$ ) include 0 and 1 values, respectively, both methods can be considered equivalent, confirming the satisfactory trueness of the developed method.

Nevertheless, it should be pointed out that the LC-ESI-MS method developed in this work has better trueness than the electrochemical method, given that a maximum difference of 7.1% and a mean value of 3.6% were found for the samples with known content of iodine using the electrochemical method, against a 1.2% and a 0.8% for the LC-ESI-MS method.

Finally, as far as the seawater samples are concerned, the amount of iodide found in those taken near the shore is higher (124 to 166 nM) than the amount determined in samples taken off-shore (49 to 65 nM). These results agree with the results found by other authors [3,37,38] and seem to be related to a higher biological activity in spring and near shore. On the other hand, the total inorganic iodine (iodide + iodate) ranges from 382.7 to 513.5 nM, also agreeing with the expected in seawater samples [3,39,40].

#### 4. Conclusions

A new LC-ESI-MS method for the determination of iodide and iodate in saline samples has been developed on a mixed-mode liquid chromatography column, and fully characterized following the ICH [16] and IUPAC [17] guidelines. Iodide is quantified by direct injection of filtered samples, and iodate is determined as iodide after its reduction with ascorbic acid at low pH. The chromatographic method was optimized using a central composite design model, and allows the separation of iodide from the rest of ions and interferences of the sample in less than 20 min applying an isocratic method. The developed method shows good selectivity, sensitivity, reproducibility, recovery and robustness, with a linear range of four orders of magnitude and a limit of detection as low as 0.16 nM.

The applicability of the LC-ESI-MS method has been demonstrated analyzing different real saline samples and comparing the results with those obtained applying the well-established voltamperometric technique. In both cases, the amount of iodide and iodate was statistically equivalent.

In short, it can be said that the developed method allows the fast and high throughput quantification of iodide and iodate in saline matrices avoiding any sample treatment, reaching very low limits of detection and using just a few microliters of sample, outperforming the rest of published methods.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.chroma.2018.12.046>.

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